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Original Article

Sweat chloride as a biomarker of CFTR activity: Proof of concept and ivacaftor clinical trial data

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Abstract

Background: We examined data from a Phase 2 trial {NCT00457821} of ivacaftor, a CFTR potentiator, in cystic fibrosis (CF) patients with a *G551D* mutation to evaluate standardized approaches to sweat chloride measurement and to explore the use of sweat chloride and nasal potential difference (NPD) to estimate CFTR activity.
Methods: Sweat chloride and NPD were secondary endpoints in this placebo-controlled, multicenter trial. Standardization of sweat collection, processing, and analysis was employed for the first time. Sweat chloride and chloride ion transport (NPD) were integrated into a model of CFTR activity.
Results: Within-patient sweat chloride determinations showed sufficient precision to detect differences between dose-groups and assess ivacaftor treatment effects. Analysis of changes in sweat chloride and NPD demonstrated that patients treated with ivacaftor achieved CFTR activity equivalent to approximately 35%–40% of normal.
Conclusions: Sweat chloride is useful in multicenter trials as a biomarker of CFTR activity and to test the effect of CFTR potentiators.

Keywords: Cystic fibrosis; Nasal potential difference; Variance; Sweat test

1. Introduction

Cystic fibrosis (CF) is a multisystem disease caused by mutations in the gene coding for the cystic fibrosis transmembrane conductance regulator (CFTR) protein, which regulates ion flux at the surface of certain epithelial cells [1]. Restoring defective CFTR ion transport is a promising therapeutic approach under evaluation in individuals with CF, creating a need for suitable biomarkers of CFTR activity. In the clinical setting, the most common methods to assess CFTR function are sweat chloride concentration and nasal potential difference (NPD) [2]. Sweat chloride is appealing as a biomarker because abnormalities occur early, it can be measured consistently in patients at any age, and sweat glands do not appear to be susceptible to secondary damage

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from the disease process (unlike other tissues such as the lung and gastrointestinal tract) [2]. Sweat chloride is widely used as a diagnostic tool for CF [3] and is substantially more convenient and less costly to perform than NPD.

Ivacaftor is a CFTR modulator that potentiates the chloride transport of several CF disease-causing forms of CFTR, including *G551D*-CFTR [4,5]. The *G551D-CFTR* mutation, termed as gating mutation, results in a protein with severely restricted channel opening but normal cell membrane expression levels [6]. A Phase 2, dose-ranging trial of ivacaftor was conducted in patients with CF who have the *G551D* mutation on at least 1 *CFTR* allele [7]. The primary objective of this trial was to evaluate safety; however, secondary endpoints included exploratory biomarkers of CFTR function: sweat chloride concentrations and NPD testing. Patients receiving ivacaftor achieved significant reductions in sweat chloride when evaluated both within-patient (change from baseline) and placebo controls.

Despite the development of best practices for sweat chloride testing in the diagnosis of CF [8], multiple published reports in the past 10 years have concluded that consistency and reliability are poor across laboratories. Assessments performed worldwide revealed numerous concerns, including lack of conformity to guidelines, inadequate quality control measures, low numbers of tests performed yearly, and inconsistencies in minimum sweat volumes, iontophoresis solutions, and reference ranges [9,10].

In anticipation of the need for clinical testing of CFTR potentiators, such as ivacaftor, in multicenter trials, the Cystic Fibrosis Foundation Therapeutics Inc.—Therapeutic Development Network, in collaboration with Vertex Pharmaceuticals, developed standard operating procedures for sweat collection using the Macroduct™ system (Wescor/ELITech Group, Logan, UT) with the aim of minimizing variability across sites. Improvements included standardization of protocols for: 1) sweat collection, 2) frozen storage, 3) overnight transport to the central laboratory for analysis, and 4) uniform training of personnel at all sites.

This Phase 2 ivacaftor study was the first to utilize these new protocols. We examined reliability in sweat chloride data by determining within- and between-patient variances. Finally, published sweat chloride and NPD values were used to estimate CFTR activity.

2. Methods

2.1. Study design

The design of this ivacaftor Phase 2 study is fully described elsewhere [7]. Briefly, the study was a randomized, double-blind, placebo-controlled, multicenter trial conducted in 2 parts. In Part 1, 20 patients with CF were randomized to receive either ivacaftor every 12 h at doses of 25 mg, 75 mg, or 150 mg, or matching placebo for 14 days in a crossover, dose-ascending format. In Part 2, 19 new patients were randomized to receive ivacaftor every 12 h at doses of 150 mg or 250 mg, or placebo for 28 days. The study enrolled adult patients with CF who had the *G551D* mutation on at least 1 *CFTR* allele and a forced expiratory volume in 1 s (FEV_1) of at least 40%. The primary study objective was to evaluate the safety and tolerability of ivacaftor. Secondary endpoints included sweat electrolyte concentration, NPD, pulmonary function testing, and patient-reported health-related quality of life.

During Part 1, sweat and blood samples were collected at screening and on days 1, 7, and 14 of each treatment period, and at a follow-up visit. NPD testing was performed on days 1 and 14 of each treatment period and at a follow-up visit. In Part 2, sweat samples were collected at screening and on days 1 (baseline), 3, 14, 21, and 28 of the treatment period and at a follow-up visit; NPD was performed on days 1, 14, and 28.

2.2. Sweat chloride and NPD methodology

We adapted sweat collection methods previously used in multicenter clinical studies. We developed standardized approaches for sweat collection and shipping to a central laboratory for analysis, and examined the effects of freezing and storage of sweat samples (see online supplement). Between- and within-patient variances were computed. NPD testing was performed as previously described [7]. Training and qualification of NPD operators are described in the online supplement.

2.3. NPD as a surrogate for CFTR activity and % CFTR estimation

We used three approaches to estimate the degree of improvement in CFTR activity among patients treated with ivacaftor based on sweat chloride concentrations and NPD measurements: 1. sweat chloride measurements alone, 2. NPD measurements alone, and 3. sweat chloride and NPD measurements considered together with NPD as a surrogate of CFTR activity.

2.3.1. Sweat chloride measurements alone

In our first approach, we calculated the percentage change in sweat chloride during the Phase 2 study, with pooling of treatment groups where appropriate.

2.3.2. Nasal potential difference alone

In our second approach, we examined a linear relationship between NPD and CFTR activity. We defined the absence of CFTR activity (zero activity) as the chloride-free-plus-isoproterenol NPD response in pancreatic insufficient (PI) CF patients [11–13]. Although very minimal activity may be maintained in these patients, it is likely below the limit of detection of this assay. We defined full CFTR activity (100%) as the chloride-free-plus-isoproterenol response in control, non-CF individuals [14]. Using these definitions, % CFTR activity for any group of patients can be calculated by the following equation:

- % CFTR activity = (X − PI CF group) / (control − PI CF group) * 100, where
  - X is the chloride-free-plus-isoproterenol response for the sample group;
  - PI CF group is the chloride-free-plus-isoproterenol response in patients with CF who are PI (no measurable CFTR activity); and

- Control is the chloride-free-plus-isoproterenol response for the control group—patients without CF (100% CFTR activity).

To estimate the effects of ivacaftor on CFTR activity, data from the Phase 2 trial were analyzed retrospectively according to the equation above. Reference values (control and PI CF) for calculating % CFTR activity in patients treated with ivacaftor were drawn from subjects evaluated during the pre-trial NPD technician qualification program. NPD technicians at participating centers were trained and tested; mean data from CF (n = 16) and control (n = 17) subjects tested during this period are shown in supplemental Fig. E1.

2.3.3. Sweat chloride and NPD measurements considered together with NPD as a surrogate of CFTR activity

In our third approach, we used NPD measurements as a surrogate for CFTR activity. The respiratory tract is a relevant disease-affected target organ for intervention, and thus changes in CFTR activity as measured by the NPD in the respiratory tract (across numerous phenotypes and studies) were chosen for comparison with sweat chloride. In this approach, we expanded the % CFTR activity analysis by examining published studies that reported data for NPD and sweat chloride in CF patients with varying degrees of disease severity and control individuals [15–24].

3. Results

3.1. Sweat chloride training, freezing and stability

A total of 36 sweat chloride collection technicians from 15 sites in 3 countries were successfully trained and participated. The training included in-person sessions with observers who certified the individual technicians. Each trainee had to send in a valid collection specimen to the central lab prior to being approved for the trial. As shown in supplemental Fig. E2, sweat electrolytes were not affected by overnight shipping. In addition, sweat chloride and sodium were stable over 18 months when stored at −70 °C (supplemental Fig. E3).

3.2. Variances in sweat chloride measures

Within-patient and between-patient variances for sweat chloride values from Parts 1 (n = 20) and 2 (n = 19) of the Phase 2 ivacaftor trial are shown in Fig. 1 as a function of dose. Among patients treated with placebo, within-patient variance in sweat chloride (19.3 $[mmol/L]^2$ for Part 1 and 19.8 $[mmol/L]^2$ for Part 2), was generally comparable to between-patient variance (36.7 $[mmol/L]^2$ in Part 1 and 3.1 $[mmol/L]^2$ in Part 2). Among all patients treated with ivacaftor, within-patient variance was low (65.2 $[mmol/L]^2$ in Part 1 and 42.1 $[mmol/L]^2$ in Part 2) relative to the magnitude of the treatment effects (e.g., the median change of −59.5 mmol/L with the 150 mg ivacaftor dose) in the range of dosing levels studied here. Between-patient variances were high (452.1 $[mmol/L]^2$ in Part 1 and 358.3 $[mmol/L]^2$ in Part 2) for all patients treated with ivacaftor. The intraclass correlation coefficient (ICC; variance between patients/total variance) provides an estimate of the contribution of between-patient variance. For all patients treated in the ivacaftor study, the ICC indicated that the majority of variance came from differences between patients (sweat chloride ICC = 0.92 in Part 1 and 0.94 in Part 2).

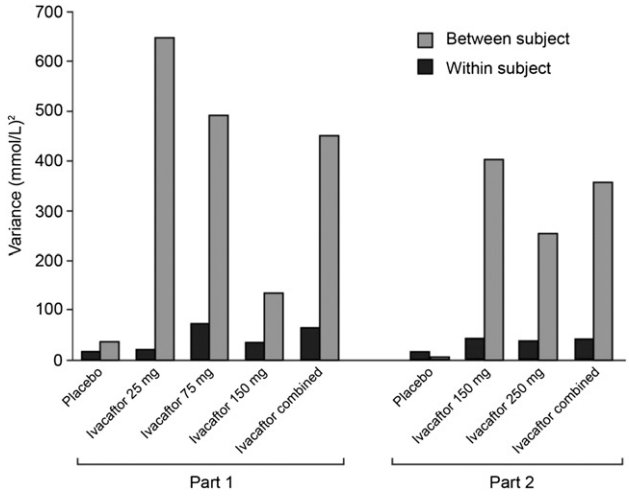

Fig. 1. Within- and between-patient variance in sweat chloride over repeated measures by dose of ivacaftor and placebo. Data are shown for Parts 1 and 2 of the clinical trial. Within-patient variances were similar across treatment groups, indicating a consistent treatment effect in given individuals. Between-patient variances were high in the treatment groups, indicating variable effects of ivacaftor in different individuals.

3.3. Ivacaftor treatment, sweat chloride, NPD, and CFTR activity

We took three approaches to estimate the degree of improvement in CFTR activity among patients treated with ivacaftor based on sweat chloride concentrations and NPD measurements.

3.3.1. Sweat chloride measurements alone

First, we calculated the percentage change in sweat chloride during the Phase 2 study. The percentage provides a linear approximation of functional gain in chloride ion transport through the CFTR channel (Table 1). Using this approach, patients treated with ivacaftor experienced improvements in chloride transport ranging from 29% to 47% (compared with 2% for the placebo arm).

3.3.2. Nasal potential difference alone

We used changes in NPD to determine CFTR activity with ivacaftor treatment. The zero-chloride plus isoproterenol NPD response was converted to a percentage of *normal* CFTR activity (see Methods for equation). The zero-chloride plus isoproterenol responses obtained on Day 14 suggested that patients treated with ivacaftor experienced gains in CFTR activity in the range of 15% (at the 25 mg dose) to 43% (at the 250 mg dose) (Table 2).

Table 1
Actual and percentage change from baseline in sweat chloride concentrations in the Phase 2 ivacaftor trial.

Ivacaftor dose	Phase 2 time point	n	Mean baseline sweat chloride from Phase 2 trial (mmol/L)	Change from baseline in sweat chloride (mmol/L)	% change from baseline
25 mg	Part 1, Day 14 (Group A)	8	104.9	−32.9	31%
75 mg	Part 1, Day 14 (Groups A & B)	14	100.7	−40.4[a]	40%
150 mg	Parts 1 & 2, Day 14	16	98.8	−46.0[b]	47%
250 mg	Part 2, Day 14	7	94.9	−27.6	29%
Placebo	Parts 1 & 2, Day 14	12	102.9	2.0[a]	2%

[a] Change from baseline available for 13 patients and 11 patients in the 75 mg and placebo groups, respectively.
[b] For 150 mg dose, change from baseline in sweat chloride was −42.3 and −52.6 mmol/L for Part 1 and Part 2, respectively.

3.3.3. Sweat chloride and NPD measurements considered together with NPD as a surrogate of CFTR activity

In our third approach, sweat chloride and NPD findings were related to historical data on CFTR activity among different phenotypic manifestations of CFTR-related disease (Table 3). In Fig. 2, mean sweat chloride concentrations across a range of CFTR disease severity categorizations (including controls without CF) are plotted against the zero-chloride-plus-isoproterenol responses (see Methods for details). NPD values from different populations (starting with the most severely affected: CF patients who are PI, pancreatic sufficient, carriers of known and atypical mutations) were obtained from more than 20 published patient cohorts (Table 3) [15–24] across the spectrum of mutation severity and disease phenotypes.

Mean baseline CFTR activity for the ivacaftor study population was comparable to that seen in patients with CF who are PI and display minimal chloride ion transport capacity. When graphed as a function of sweat chloride and NPD, CFTR activity calculated for patients treated with ivacaftor was essentially superimposable upon the CFTR activity curve determined by genotype–phenotype relationships (Fig. 2). This suggests that changes in NPD and sweat chloride with ivacaftor are similar to those seen with different CFTR phenotypes (and corresponding genotypes). To further examine effects of ivacaftor on CFTR activity, we plotted change in sweat chloride vs. change in chloride-free-plus-isoproterenol NPD data for individual patients. As shown in supplementary Fig. E4, placebo patients showed very little change in sweat chloride or NPD, whereas patients with ivacaftor showed effects consistent with increases in CFTR activity.

Table 2
NPD chloride-free-plus-isoproterenol responses and conversion to % CFTR activity at Day 14 in the Phase 2 ivacaftor trial.

Ivacaftor dose	Source	n	NPD chloride-free + iso response, mV (range)	NPD response as % CFTR activity[a]
25 mg	Part 1, Day 14 (Group A)	8	1.0 (−5.3 to 9.0) Placebo = 2.3 (−1.5 to 5.0)	3.5/22.6 15%
75 mg	Part 1, Day 14 (Groups A and B)	16	−2.5 (−16.5 to 4.5) Placebo = 0.9 (−5.5 to 5.0)	7/22.6 31%
150 mg	Part 1 Day 14 (Groups A and B)	8	−2.1 (−7.8 to 4.8) Placebo = 0.9 (−5.5 to 5.0)	6.6/22.6 29%
150 mg	Part 2, Day 14	8	−2.5 (−7.5 to 0.3) Placebo = 3.5 (0.3 to 7.0)	7/22.6 31%
250 mg	Part 2, Day 14	7	−5.3 (−12.0 to 1.7) Placebo = 3.5 (0.3 to 7.0)	9.8/22.6 43%

Definition of abbreviations: CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; NPD = nasal potential difference.
[a] The reference values from the ivacaftor qualification study for chloride-free-plus-isoproterenol response were −18.1 mV (control non-CF) and 4.5 mV (PI-CF). % CFTR activity was calculated as described in the Methods.

4. Discussion

Using data from a Phase 2 trial of ivacaftor, we have demonstrated the utility of sweat chloride as a biomarker for evaluating changes in CFTR activity in the clinical trial setting. Similar to NPD response, sweat chloride levels have been observed to respond rapidly to treatment with ivacaftor, making them attractive biomarkers for monitoring a biologic effect shortly after treatment initiation. With a short training program and standardized protocols, technicians were able to collect sweat chloride measurements with good reliability across multiple study sites. Importantly, changes in sweat chloride concentrations reflected active vs. placebo treatment and enabled the evaluation of dose-specific effects. This was the first validation of standardized protocols and procedures for testing sweat chloride as an outcome measure in a multicenter clinical trial of a CFTR potentiator.

We found low variance in sweat chloride concentrations (both within- and between-patient) in patients treated with placebo. Low variance was also present for the within-patient measures of sweat chloride following ivacaftor treatment, whereas between-patient variance was high. The higher between-patient variance, when compared with within-patient variance, suggested that patients responded differently to ivacaftor. The low within-patient variance during treatment indicates that the response in a given patient was relatively stable over the treatment period.

Given that ivacaftor is the first agent associated with large magnitude reductions in sweat chloride in patients with CF, we found limited published data to provide direct context for these findings. Among the few published studies of agents designed to modulate CFTR activity, the majority did not measure sweat chloride [25], one reported very small changes (5 mmol/L after 10 weeks of lithium chloride) [26], and another did not measure variability [27].

Data from a multicenter clinical trial evaluating changes in sweat chloride and NPD in patients with CF treated with a CFTR trafficking modulator, CPX (adenosine A1 receptor 8-cyclopentenyl-1,3-dipropylxanthine), are generally consistent with our findings [28]. Although no efficacy was seen with CPX, the authors evaluated within- and between-patient variances across their 4 participating study sites. Our variances were lower than those in the CPX study, perhaps because of standardization of techniques. The variances in the patients treated with placebo in our study may provide a benchmark for future studies.

Possible explanations for the large variability seen between patients in the ivacaftor treatment groups include differences between the time of drug administration and the time of sweat chloride collection, differences in the amount of CFTR activity correction in individual patients, and variability in ivacaftor exposure between patients. Possible reasons for differences in the amount of ion transport correction include the specific non-*G551D* mutation in the other allele, the relative quantity of CFTR channels at the cell surface, and/or the role of modifier genes impacting ion balance. The importance of these differences in degree of sweat chloride changes and how these result in a clinical benefit is not known.

The change in sweat chloride across dosing groups suggests a non-linear relationship between sweat chloride and CFTR activity. To estimate the degree of CFTR functional activity in patients treated with ivacaftor, we related sweat chloride to NPD. We included patient data from multiple published studies of children and adults (excluding infants) with varying CF phenotypes to better characterize the relationship between genotype/phenotype and CFTR activity.

The treatment effect with ivacaftor in all four dosing groups was closely aligned with the CFTR activity observed in previously published studies, which supports a consistent relationship between CFTR activity, disease manifestations, and response to a CFTR potentiator. Patients with CF treated with ivacaftor for 14 days achieved CFTR activity levels corresponding to approximately 35% (with 75 mg and 150 mg) and 40% (with 250 mg) of non-CF controls. This magnitude of improvement is also consistent with that predicted in a previous publication based on in vitro research with ivacaftor [4]. Investigators tested transmembrane potential differences using primary cultures of human bronchial endothelial (HBE) cells from patients with CF (G551D/F508del) and controls without CF. When stimulated, the potential change in G551D/F508del HBE cells was approximately 5% of that seen with non-CF HBE cells, and in the presence of ivacaftor, rose to approximately 48% of control HBE cells.

The model of sweat chloride and NPD presented here is consistent with sweat chloride biomarker data drawn from disparate published trials in patients with varying degrees of CF disease severity. It brings an additional dimension to sweat chloride as a biomarker by demonstrating that estimates of CFTR activity that integrate NPD responses produce findings comparable to those obtained with sweat chloride alone. Finally, the close alignment between data from patients treated with ivacaftor and historical controls provides verification of the role of ivacaftor in altering chloride ion transport.

4.1. Study limitations

Our study has several important limitations. It is likely that a CFTR potentiator such as ivacaftor may differentially impact CFTR activity across tissue types. In this study we examined the sweat gland and the nasal epithelium. Interestingly, the changes in sweat chloride (expressed as percentage change from baseline) paralleled the changes in NPD. Data from the literature (Fig. 2) also showed that sweat chloride and NPD have, as a first approximation, a near linear relationship across clinical phenotypes. A recent study reported a linear relationship between adrenergically mediated sweat secretion among patients with CF, CF carriers, and normal individuals [29]. The sweat secretion test could not, however, detect a difference between pancreatic-sufficient and -insufficient patients with CF and did not find a difference in most patients with CFTR-related disorders, whereas sweat chloride concentration and NPD show a difference between these groups. There is evidence to suggest that early sweat chloride response may indicate a higher likelihood of longer-term lung function change (up to Week 16) in patients treated with a CFTR potentiator [31]. While while another study found no correlation between changes in sweat chloride concentration and clinical outcomes [30].

Our analyses were also limited by the small number of patients in the study. With a larger number of patients, between-site variability could have been examined. Variability in NPD testing is a concern in general and is reflected in the values for the controls (non-CF or normal patients) reported in the publications in Table 3. The degree of variability from respected investigators suggests that caution should be exercised when choosing a single value, but the value of −18 mV reported here was comfortably in the center of the range of published values.

5. Conclusion

In summary, we developed standardized procedures to measure sweat chloride in multicenter trials of an orally-administered CFTR potentiator. Within-patient variance in sweat chloride was shown to be low for ivacaftor and placebo-treated patients in this first trial utilizing these new procedures. Sweat chloride response to treatment was related to ivacaftor dose. These findings support the use of sweat chloride testing, a convenient and reproducible biomarker of CFTR activity, in clinical trials of CF evaluating CFTR potentiators. We also suggested an approach to estimate change in CFTR activity using sweat chloride and NPD values taken from the ivacaftor trial and other published studies. This model demonstrated that in response to treatment with ivacaftor, patients achieved a restoration of CFTR activity in the range of 35%–40% of that seen in control patients without CF. Further exploration of the relationship between sweat chloride concentrations, ion transport (as measured by NPD), and clinical outcome measures such as lung function with CFTR modulators may provide insights into subtleties of ion channel function.

Conflict of interest statement

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Table 3
References for CFTR activity analysis.

Reference	Disease phenotype	n for sample cohort	Sample cohort description	Sweat chloride for sample cohort (mmol/L)	Sample cohort Cl-free + iso response (mV)	Control (non-CF) zero Cl + iso response (mV)	CF-PI Cl-free + iso response (mV)	% CFTR calculation	Control (non-CF) group defined
Wilschanski [15]	Atypical CF; 9 were PS and 2 were PI	11	Patients with atypical CF presentation or CF suspected	79 (mean from Table 1)	0.0	–12.0	3.0	20	Control group was normal individuals or those with non-CF lung disease (n = 50) and typical CF patients (n = 31)
Wilschanski [16]	Non-CF control	25	No family history of CF or evidence of pulmonary or pancreatic disease	20	–29.0	–29.0	4.0	100	
	CF/obligate heterozygote	21	Fathers or male siblings of CF patients; confirmed by genotype	26	–23.0	–29.0	4.0	81.8	
	Incidental heterozygote	6	Healthy control patients with *CFTR* mutation on 1 allele	34	–27.0	–29.0	4.0	93.9	
	CBAVD-0 with no mutations	6	Men with CBAVD	22	–22.0	–29.0	4.0	78.8	No family history of CF nor evidence of pulmonary or pancreatic disease
	CBAVD-1	18		44	–12.0	–29.0	4.0	48.5	
	CBAVD-2	36		54	–8.0	–29.0	4.0	36.4	
	CF PS	24	Men with diagnosed CF	73	2.0	–29.0	4.0	6.1	
	CF PI	26		102	4.0	–29.0	4.0	0	
Gilljam [17]	R117H (PS); 5 of these are 7 t and one is 5 t	6	Patients with CF diagnosis in adulthood	72; mean from Table 4	–5.4	–24.6	3.6	32	Controls not further defined
	CF PS	27		66	1.7	−24.6	3.6	6.7	
Bishop [18]	CF PS	56		68.7	–0.5	–24.6	3.5	14.2	

			Patients with idiopathic pancreatitis						50 healthy controls not further defined
	Obligate heterozygote	16		30.6	−17.7	−24.6	3.5	75.4	
	Pancreatitis, 2 mutations	6		41	−7.1	−24.6	3.5	37.7	
	Pancreatitis, 1 mutation	18		34.4	−19.5	−24.6	3.5	81.8	
Lebecque [19]	Intermediate sweat chloride with 2 mutations	10	Children with borderline sweat chloride levels	39.4	−9.4			72	Not defined
Segal [20]	Recurrent pancreatitis with abnormal NPD	7	Recurrent acute pancreatitis	44	−1.00	−10	2.5	24.1	50 control patients and 50 CF patients were used for comparison
Wang [21]	Chronic rhinosinusitis patients with 1 mutation	9	Chronic rhinosinusitis patients with 1 CF mutation; adults with nasal or sinus symptoms × 8 wks or 4 episodes of recurrent symptoms in past year	37.4	−11.0	−15.8	5.2	77	10 volunteers not having CF who had <10 d/y of signs and symptoms of rhinosinusitis, matched for race, age range, geographic region, and socioeconomic status
Pradal [22]	CBAVD	12	Patients with CBAVD and mixed # of mutations (none in 4; 1 in 7 and 2 in the final)	39	−6			89.6	Controls were patients admitted to the center with other well documented diseases or healthy volunteers
Wallace [23]	R553X/R117L	2		80.5	−1.75	−15.7	−0.8	16	12 young people without CF
	F508/F508	30		121	−1.3		−0.8		
Walker [24]	A455E	5	Mild pulmonary disease	80	4.0	−12.0	5	5.8	10 normal patients with no history of respiratory disease or family history of CF
	G542X, all PI	5		95	2.0	−12.0	5	17.6	

Definition of abbreviations: CBAVD = congenital bilateral absence of the vas deferens; CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; NPD = nasal potential difference; PI = pancreatic insufficient; PS = pancreatic sufficient.

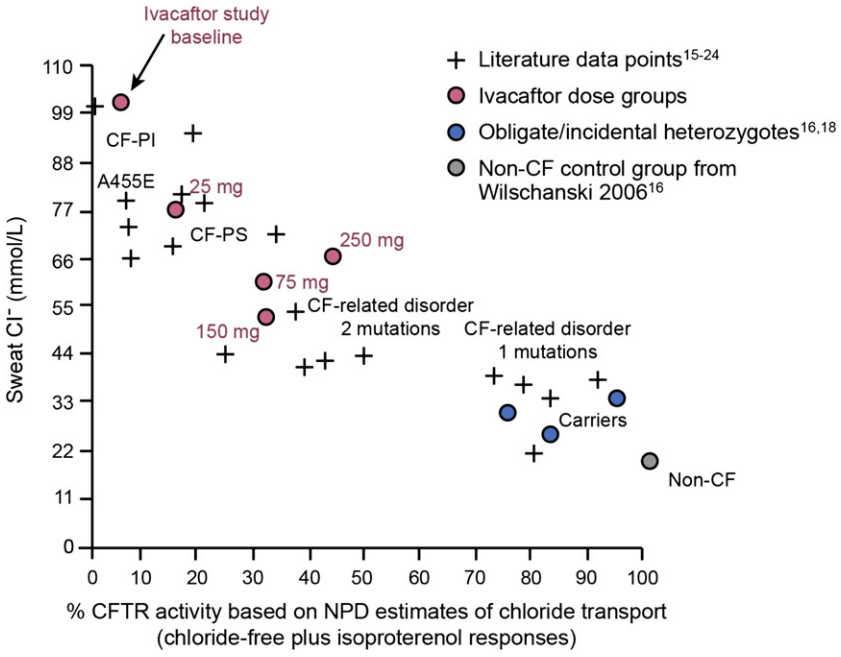

Fig. 2. Sweat chloride levels vs. CFTR activity in patients with varying CF genotypes and patients treated with ivacaftor at varying dose levels.*% CFTR activity with ivacaftor was estimated using NPD measurements taken at the same time as the sweat chloride. Data are shown for 22 cohorts reported in 10 published studies [15–24], described and tabulated in Table 3.

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Author contributions

FJA, FVG, CLO, SMR, JPC, MWK, HEH, SLH, PWC, BWR, and MAA contributed to the conception of the analyses and hypotheses presented in the paper. FJA, JZ, AJS, QD, SMR, MKW, HEH, SLH, BWR, PWC, and MAA were involved in data collection and sample testing or provided clinical information that was essential to the paper. FJA, FVG, JZ, AJS, QD, SLH, and BWR analyzed data or performed statistical analyses. All authors made substantial contributions to the writing and revisions of the paper and provided approval to submit. FJA had primary responsibility for the final content.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jcf.2013.09.007.

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